

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Jon A. Wolff,)
Vladimir S. Trubetskoy,)
Sean D. Monahan, James E. Hagstrom,)
Paul M. Slattum, Vladimir G. Budker,)
Aaron G. Loomis)
Serial No.: 09/328,975)
Filed: 6/9/99)
Group Art Unit: 1632)

Examiner: Richard Schnizer

For: Charge Reversal of Polyion Complexes

DECLARATION UNDER 37 C.F.R. §1.132

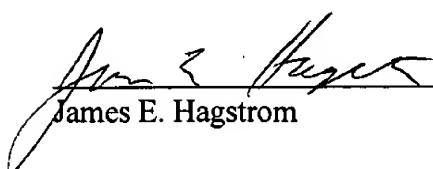
Assistant Commissioner for Patents
Washington, DC 20231

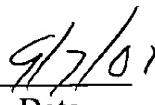
Dear Sir:

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the captioned application.
2. Applicants' process was conceived prior to the effective date of the Office Action prior art references.
3. We developed our recharging process with due diligence from conception to the filing of our application.
4. Photocopies of my personal laboratory notebook pages showing nucleic acids recharged with histone and then re-charged with liposomes dated March and June, 1994 accompany this Declaration.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


James E. Hagstrom


Date
9/7/01

3/28/

RAT Myotube Transfection

↙ ALL DNA in pH 8.5 tris

- 1> 5 ug pBSRLUX 5ug/plate
- 2> DNA + Lipofectin 2.5 λ /plate
- 3> DNA + Lipofectin 15 λ /plate
- 4> DNA + Lipo (2.5 μ g/plate) + NLS-H1 (0.5 μ g/plate)^(1,2)
- 5> DNA + Lipo (2.5 μ g/plate) + NLS-H1 (1.5 μ g/plate)^(3,4)
- 6> DNA + Lipo (2.5 μ g/plate) + NLS-H1 (0.5 μ g/plate) + MAT-H1 (0.5 μ g/plate)^(2,3)^(CB)
- 7> DNA + Lipo (2.5 μ g/plate) + NLS-H1 (0.5 μ g/plate) + MAT-H1 (1.5 μ g/plate)^(4,5)^(CB)
- 8> DNA + Lipo (2.5 μ g/plate) + NLS-H1 (1.5 μ g/plate) + MAT-H1 (0.5 μ g/plate)^(2,3)^(CB)
- 9> DNA + Lipo " " + NLS-H1 (1.5 μ g/plate) + MAT-H1 (1.5 μ g/plate)^(3,4)^(CB)
- 10> DNA + Lipo " " + NLS-H1 (1.5 μ g/plate) + MAT-H1 (1.5 μ g/plate)^(3,4)^(CB)
- 11> DNA + Lipo " " + NLS-H1 (1.5 μ g/plate) + MAT-H1 (1.5 μ g/plate)^(3,4)^(CB)
- 12> DNA + Lipo " " + NLS-H1 (1.5 μ g/plate) + DOPE (60 λ /plate)^(6,8)^(CB)
- 13> DNA + Lipo " " + NLS-H1 (1.5 μ g/plate) + DOPE (60 λ /plate)^(6,8)^(CB)
- 14> [DNA + NLS-H1 (1.5 μ g)] + DOPE (60 λ /plate)
- 15> [DNA + NLS-H1 (1.5 μ g) + MAT-H1 (0.5 μ g)] + DOPE (60 λ /plate)
- 16> [DOPE + NLS-H1 (1.5 μ g)] + DNA (5ug/plate)
- 17> [DOPE + NLS-H1 (1.5 μ g) + MAT-H1 (0.5 μ g)] + DNA
- 18> DNA 5ug/plate + DOPE 60 λ /plate

10 Day Old Myotubes -

- 1> DNA 6ug/plate) + Lipo (2.5 μ g/plate)
- 2> DNA " " + " " + NLS-H1 (1.5 μ g/plate)
- 3> DNA " " + Lipo " " + NLS-H1 " " + MAT-H1 (0.5 μ g/plate)

* For all samples except 23-32

- Add DNA (10 μ g/2 ml media) in 100 μ l Tris 8.5 + Protein
 ↓ 15° nt

Add Lipofectin

↓ 30° nt

Add to 1.5 ml on each ϕ 35 mm dish

5

5:28

MEAS TIME(S) 31.0

SAMPLE RLU
1 H₂O Blk
2 543
3 9204 H Lucifer
4 87593 5A Lucifer

5 > 5 μg/plate pBSRLUX
6 2385
7 44422 > DNA + 2.5 μg/plate Lipofectin
8 28742

9 227053 > DNA + Lipo.
10 415636 1.5μg/plate

11 16825 > DNA + Lipo + NLS-H1 (0.5μg/plate)
12 7301 (2.5)

13 727847 > DNA + Lipo + NLS-H1 (2μg)
14 727847 (1.5μg/plate)

15 56092 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg/plate)
16 10582 (0.5μg/plate) (0.5μg/plate)

17 41314 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg)
18 23834 (1.5μg) (1.5μg/plate)

19 18100 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg)
20 31813 (1.5μg) (1.5μg)

21 20410 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg)
22 32253 (1.5μg) (1.5μg)

23 2381512 > DNA + Lipo + NLS-H1
24 939243 (2μg/plate)

25 5892126 > DNA + Lipo + NLS-H1
26 3102207 (4.5μg/plate)

27 182370 [DNA + NLS-H1] + DOPE 60 μg/plate
28 331964 (1.5μg)

29 225842 [DNA + NLS-H1 + MAT-H1] + DOPE
30 248196 (1.5μg) (0.5μg)

31 132754 [DOPE + NLS-H1] + DNA
32 119066 (1.5μg)

33 406366 [DOPE + NLS-H1 + MAT-H1] + DNA

34 240774 [DNA + DOPE] 60 μg/plate

35 87183 [DNA + DOPE] 60 μg/plate

36 18896

* MAT-H1 - purified via Ni-NTA agarose and then Cibacron Blue agarose

i.e., loaded onto a C.B. column at 150 mM NaCl + eluted off with 50 mM increasing NaCl step gradient

- checked on 12% SDS-PAGE
- concentrated single band containing (MAT-H1) elution
- contained 10 μg protein (cone)

- Results - Appear that CB purified protein inhibits transfectability

* Does it bind or retard DNA in a gel-shift assay

6/7/94

43

PS/PE expt #2

- 1 > pBSR64CUP (3ug) + PS2.5¹⁸² + NLS-1+1 (2ug) 8λ (300 DNA/prof) + (200 Liposome)
- 3 > DNA + PS2.5¹⁸² + " (4ug) 16λ
- 5 > DNA + PS2.5¹⁸² + NLS 111 (6ug) 24λ
- 8 > DNA + PS5¹⁸² + " (2ug) 8λ
- 9 > DNA + PS5¹⁸² + " (4ug) 16λ
- 11 > DNA + PS5¹⁸² + " (6ug) 24λ
- 14 > DNA + PS10¹⁸² + " (2ug) 8λ
- 15 > DNA + PS10¹⁸² + " (4ug) 16λ
- 17 > DNA + PS10¹⁸² + " (6ug) 24λ
- 19 > DNA + PS2.5³⁶² + " (4ug) 16λ
- 21 > DNA + PS2.5³⁶² + " (6ug) 24λ
- 23 > DNA + PS2.5³⁶² + " (4ug) "
- 25 > DNA + PS5³⁶² + " (6ug) "
- 27 > DNA + PS5³⁶² + " (4ug) "
- 28 > DNA + PS10³⁶² + " (6ug) "
- 30 > DNA + PS10³⁶² + " (4ug) "
- 31 > DNA + PS10³⁶² + " (6ug) "
- 33 > DNA + ~~PS10~~ Lipofectin 24λ (300 DNA) + (300 Liposome)
- 35 > DNA + ~~PS10~~ Lipofectin 24λ (300 DNA) + (300 Liposome)

Protocol

- Add DNA + protein (300d optimum) 15' at rt
- Add Liposomes in (200d optimum) 15' at rt
- wash cells 1X in optimum
- Add complexed to 2ml optimum on cells
- change media after 3-4 hr
- incubate at 37°C for ~48 hrs
- harvest cells
- lux assay

• 3T3

RLU
 1 1191 > DNA + PS2.5 + NLS-H1
 2 466 (18A) (2ug)

3 66525 > DNA + PS2.5 + NLS-H1
 4 107400 18A 4ug

5 2181385 > DNA + PS2.5 + NLS-H1
 6 1997461 18A 6ug

7 485 > DNA + PS5 + NLS-H1
 8 487 18A (2ug)

9 843961 > DNA + PS5 + NLS-H1
 10 799857 18A (4ug)

11 2443514 > DNA + PS5 + NLS-H1
 12 1974928 18A 6ug

13 686 > DNA + PS10 + NLS-H1
 14 665 18A (2ug)

15 424442 > DNA + PS10 + NLS-H1
 16 31953 18A (4ug)

17 1938695 > DNA + PS10 + NLS-H1
 18 1779958 18A 6ug

19 366265 > DNA + PS2.5 + NLS-H1
 20 649356 18A (4ug)

21 2776187 > DNA + PS2.5 + NLS-H1
 22 1149081 18A (6ug)

23 1596987 > DNA + PS5 + NLS-H1
 24 1792688 18A (4ug)

25 2789949 > DNA + PS5 + NLS-H1
 26 3353918 18A (6ug)

27 270468 > DNA + PS10 + NLS-H1
 28 236696 18A (4ug)

29 2482591 > DNA + PS10 + NLS-H1
 30 2774275 18A (6ug)

31 2890371 > DNA + PS10 + NLS-H1
 32 2966735 18A (9ug)

33 273358 > DNA + PS10 + NLS-H1
 34 285150 18A (6ug)